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CONTAMINATION AND SCREENING LEVEL TOXICITY OF SEDIMENTS FROM REMEDIATED AND UNREMEDIATED WETLANDS NEAR SYDNEY, AUSTRALIA

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Abstract—The present study assessed contamination and toxicity of sediments from seven remediated and remnant wetland sites within Sydney Olympic Park, Australia, and four unremediated sites adjacent to its boundary using chemical analysis and a luminescent bacterial biosensor assay (*Escherichia coli*). Concentrations of metals (Pb, Cr, Cu, Ni, Zn, Cd, and As) and persistent organic chemicals (DDT and its metabolites, dichlorodiphenyldichloroethane and dichlorodiphenyldichloroethylene; polycyclic aromatic hydrocarbons; polychlorinated biphenyls; and polychlorinated dibenzo-*p*-dioxins and dibenzofurans) in sediments and their pore-water samples were determined. Zinc concentrations were the highest of the metals in the sediments (84–618 mg/kg), and at eight sites, metal concentrations in sediments exceeded the Australian ecological trigger values for Pb, Zn, and Ni. Concentrations of organic contaminants in the sediments exceeded the trigger values at all 11 sites for DDTs, at 6 sites for polycyclic aromatic hydrocarbons, and 5 sites for polychlorinated biphenyls. Sediment samples from the four unremediated sites outside the Sydney Olympic Park had dioxin concentrations greater than 200 pg (toxic equivalency per gram). The same four sites were identified as contaminated in pore-water toxicity tests with the luminescent biosensor, generally consistent with the bioavailable fractions of the contaminants (pore-water and Tenax® extraction data), as well as dioxin levels, in the sediments. Preliminary toxicity identification and evaluation tests of the pore water from the four sites outside the park demonstrated that organic contaminants were the main cause of toxicity to *E. coli,* with no evidence that metals contributed to the toxicity of the pore water.

Keywords—Metals Organic contaminants Toxicity Pore water Toxicity identification evaluation

INTRODUCTION

Sydney Olympic Park (SOP), Australia, was remediated and developed to host the Sydney 2000 Olympic Games. Sydney Olympic Park is located in the Homebush Bay area, 17 km west of the Sydney central business district. Before development, the park site had substantial environmental pollution problems. Chemical industries, including manufacturing of pesticides (2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, and DDT), started operation in the Homebush Bay area in the late 1920s [1]. From the 1960s to the 1980s, this area was the dumping site for much of Sydney's household and industrial waste. Sydney Olympic Park was highly contaminated with metals and persistent organic pollutants prior to remediation in the 1990s [2]. The remediation of the SOP, including removal, treatment, and on-site storage of contaminated sediments and soils, was carried out in the 1990s in preparation for the Olympic Games. Some of the wetlands were created during the remediation program, while others are remnant water bodies that had undergone some remediation. The SOP wetlands are currently home to varied wildlife, including the endangered green and golden bell frog (*Litoria aurea*). To protect the biological diversity of these remnant and constructed landscapes of the park, it is advisable to investigate the potential effects such chemical contaminants may have on wildlife.

Approaches using chemical analysis and bioassays are increasingly used to assess contaminated sites. Luminescent bacterial biosensors, including Microtox® (Strategic Diagnostics) and other lux-marked biosensors, are rapid and convenient tools for determining the toxicity of contaminants in water, soil, and sediment samples [3–4]. Metabolic lux biosensors have been applied successfully to a range of organic and inorganic contaminants and shown to be sensitive, reproducible, and not limited by a narrow pH range [5–11]. As it is difficult to assess toxicity of whole sediment to various species in terms of bioavailability, pore water has often been used in toxicity screening since it is a key exposure route for contaminants to benthic organisms [12–14].

Pore-water extracts of sediments can be further evaluated using the toxicity identification and evaluation (TIE) approach to identify the toxicants responsible for the biological effects in sediments [13–15]. The TIE approach is based on the principle of sequential removal of various chemical fractions coupled with toxicity testing of the resulting fractions. When removal of a class of chemicals also removes toxicity, it is assumed that this class of chemicals is responsible for some of the toxicity observed in the original sample. The TIE manipulations

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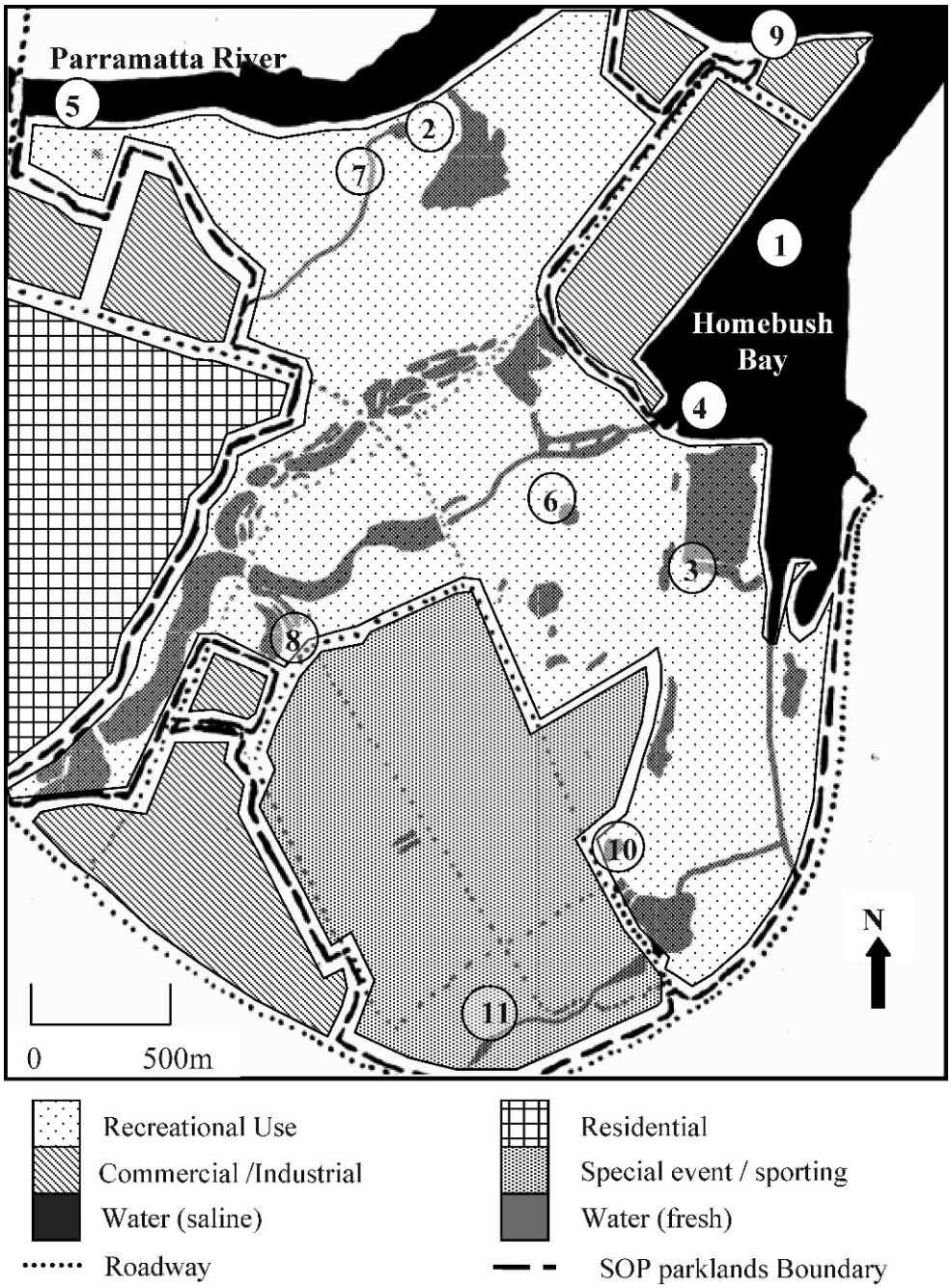

Fig. 1. Map of Sydney, Australia Olympic Park (SOP) and Homebush Bay showing water and sediment collection sites. Sites: 1 = Homebush Bay; 2 = Wharf Pond; 3 = Bicentennial Park; 4 = Haslams Mouth; 5 = Silverwater Bridge; 6 = Eastern Water Quality Control Pond; 7 = Armory; 8 = Northern Water Feature; 9 = Newington Wharf; 10 = Southern Water Quality Control Pond; 11 = Boundary Creek.

commonly performed are filtration, pH adjustment, chelation of metals with ethylenediaminetetraacetic acid or resin, oxidant reduction using sodium thiosulfate, and reversed phase chromatography using C18 columns [13,14,16]. The TIEs performed on pore water or interstitial water of marine or freshwater sediments have previously identified ammonia, metals, and organic chemicals as bioavailable toxicants [17].

The objective of the present study was to assess toxicity of sediments from the SOP using a lux bacterial biosensor (*Escherichia coli* HB101 pUCD607) in combination with chemical analysis. Various inorganic and organic contaminants—metals or metalloids (Cr, Ni, Cu, Zn, Cd, Pb, and As), DDT and its metabolites (dichlorodiphenyldichloroethane and dichlorodiphenyldichloroethylene), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs)—were analyzed in sediments and their pore-water samples. Bioavailability of organic compounds in sediments was investigated using an operational approach by Tenax® (Alltech) extraction [18]. The TIE approach was also applied to identify potential classes of chemicals in sediments that demonstrated significant toxicity.

MATERIALS AND METHODS

Study sites

A range of wetlands and streams covering approximately 175 hectares within SOP were selected as study sites (Fig. 1).

Table 1. Description of the study sites in the Sydney Olympic Park (SOP), Australia (i.e., S2, S3, S6–S8, S10, S11) and its surrounds (i.e., S1, S4, S5, S9)

		Site no.[a]	Site name	Type
Non-SOP	Unremediated	S1	Homebush Bay	Contaminated bay
		S4	Haslams Mouth	River mouth at Homebush Bay
		S5	Silverwater Bridge	Located at Parramatta River
		S9	Newington Wharf	Located at Parramatta River
SOP	Remnant	S2	Wharf Pond	Remnant wetland
		S3	Bicentennial Park	Remnant mangrove
		S7	Armory	Stream inside SOP
	Remediated	S6	Eastern Water Quality Control Pond	Storm water collection
		S8	Northern Water Feature	Constructed wetland
		S10	Southern Water Quality Control Pond	Storm water collection
		S11	Boundary Creek	Stream inside SOP

[a] Corresponding to the numbers in the location map (Fig. 1).

Some wetlands (S6, Eastern Water Quality Control Pond; S8, Northern Water Feature; and S10, Southern Water Quality Control Pond) were created during the remediation program, while some sites (S2, Wharf Pond; S3, Bicentennial Park; S7, Amory; and S11, Boundary Creek) are remnant wetlands (Table 1). Unremediated sites (S1, S4, S5, and S9) in Homebush Bay and Parramatta River, which are adjacent to but outside of the SOP boundary, were included in the study to provide some reference to archival contamination conditions in the area.

Sample processing and extraction

Composite sediment samples (1 L each) were collected using a grab sampler from each study site in and around the SOP (Fig. 1). At least 10 grab samples from the top 5 cm (estimated to be the average depth of the oxic layer), over an area of approximately 225 m^2, were collected using a 10-cm-diameter grab sampler. Collected sediment was sieved through a 2-mm sieve to remove plants and gravels on the site and was stored in clean (detergent and acid washed, solvent and deionized water rinsed) 1-L glass jars on ice for transport to the laboratory. In general, the protocols suggested by the U.S. Geological Survey [19] in the *National Field Manual for the Collection of Water-Quality Data* were used.

Pore-water samples were obtained from wet sediment samples in 50-ml glass centrifuge tubes, with a small pipe spacer in the bottom of the tube, by centrifugation at 3,500 rpm for 45 min. The pore-water samples were filtered through 0.45-μm membrane filters to remove suspended matters and were stored in a fridge at 4°C for later assaying.

Wet sediment samples were transferred into glass jars (250 ml) and freeze-dried for one week [20,21]. Persistent organic pollutants, including PAHs, PCBs, and DDTs, in the freeze-dried sediments were extracted with dichloromethane using a Dionex ASE300 accelerated solvent extractor. Briefly, the procedure was carried out as follows: 5 g of each sample were weighed into each extraction cell with a filter pad at the bottom, and the rest of the space was filled with diatomaceous earth from Dionex. Accelerated solvent extractor conditions used were as follows: Oven 100°C, pressure 10 MPa (1,500 psi), heat-up time 5 min, static time 5 min, two cycles. The extracts in collection bottles were concentrated under a nitrogen gas stream using a Dionex SE500 solvent evaporator. Final extracts were redissolved in 1 ml of hexane. The extracts were further cleaned up using solid-phase extraction silica cartridges.

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans in the sediments from the SOP were extracted using the Soxhlet method and were analyzed by high-resolution gas chromatography coupled with high-resolution mass spectrometry [22,23]. Briefly, the freeze-dried sediments were Soxhlet extracted with toluene (pesticide grade, Merck) for 72 h. Prior to extraction, 15 $^{13}C_{12}$–PCDD/F internal standards were spiked into the sediments. During the extraction, activated copper was added to remove sulfur from the samples. The extracts of the samples were concentrated using a rotary evaporator and further purified using silica and alumina columns before injection onto the instrument [23].

Chemical analysis

Analysis of heavy metals was conducted at the Commonwealth Scientific and Industrial Research Organization Analytical Service, an accredited laboratory, using standardized and validated methods. Total metals in sediment samples were determined using U.S. Environmental Protection Agency Method 3051A [24]. The finely ground sediment sample was digested in a microwave oven using a mixture of nitric acid and hydrochloric acid. The solution was then analyzed for the selected elements (As, Cr, Cu, Ni, Zn, Cd, and Pb) by inductively coupled plasma optical emission spectrometry. The instrument used was a Spectroflame Modula (Spectro Analytical Instruments).

Pore-water samples were analyzed using inductively coupled plasma mass spectrometry according to U.S. Environmental Protection Agency Method 6020A [25]. The instrument used was an Agilent 7500ce (Agilent Technologies) connected to a CETAC Technologies autosampler, which nebulizes the sample into a plasma. The samples were run at 10-fold dilutions (using reagent-grade water). Helium was added as a collision gas to remove any mass interference.

The target persistent organic pollutants included 16 PAHs (acenaphthene, acenaphthylene, anthracene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*a*]pyrene, benzo[*ghi*]perylene, benzo[*k*]fluoranthene, chrysene, dibenzo[*a,h*]anthracene, fluoranthene, fluorine, indeno[1,2,3-*cd*]pyrene, naphthalene, phenanthrene, pyrene), 11 PCB congeners (28, 52, 77, 81, 101, 118, 126, 138, 153, 169, 180), DDTs (*p,p′*-dichlorodiphenyldichloroethylene, *p,p′*-dichlorodiphenyldichloroethane, and *p,p′*-DDT) and 17 PCDD/Fs. The PAHs, DDTs, and PCBs were analyzed by gas chromatography–mass spectrometry (Agilent 6890GC-5973N mass detector) according to U.S. Environmental Protection Agency Method 8270D [26] after some modification. Target compounds were separated on a capillary column (HP-5MS, 30 m × 0.25 mm, 0.25-μm-thick stationary phase), with helium as the carrier gas, at a constant flow rate

of 1.1 ml/min. Quantitative mass spectrometric analyses were conducted by using an electron ionization source and selected ion monitoring mode. The method detection limits were 0.2 to 0.5 ng/g for chlorinated hydrocarbons and 2 to 10 ng/g for PAHs for 1 g of sediment sample.

Dioxins and furans were determined in the State Key Laboratory of Organic Geochemistry, an accredited dioxin laboratory in China, using high-resolution gas chromatography–mass spectrometry according to U.S. Environmental Protection Agency Method 8290A [27] with some modifications [22,23]. Analysis of the PCDD/Fs in the extracts was performed using a Trace GC 2000 and a Thermo Electron Finnigan MAT 95XP (Thermo Scientific) with a capillary column (CP-Sil CB/MS, 60 m × 0.25 mm internal diameter, 0.25 μm film thickness). Helium was used as the carrier gas at a flow rate of 0.8 ml/min. The sample (1 μl) was injected using the splitless injection mode. The gas chromatography temperature was programmed as follows: 90 (1 min) to 220°C (7 min) at 55°C/min, then to 275°C at 1.2°C/min, and finally to 301°C at 1.7°C/min. The gas chromatography injection port, high-resolution mass spectrometry interface, and source temperatures were kept at 250°C. The high-resolution mass spectrometry was operated in the electron impact ionization mode with its energy of 55 eV. The method detection limits were from 0.1 to 0.8 pg/g for the PCDD/F congeners. The international toxic-equivalent factors presented by the North Atlantic Treaty Organization were used to calculate the international toxic equivalency (I-TEQ) of PCDD/Fs.

Quality control and quality assurance were conducted with method blanks (solvents), spiked blanks (standards spiked into solvents), matrix spikes, and reference materials. The spike recoveries for the PCDD/F surrogate standards ranged from 78 to 95%, while those for DDTs, PCBs, and PAHs were within 80 to 120%.

Bioavailability of organic contaminants

Bioavailability of the selected organic contaminants in the sediments was assayed using the Tenax extraction method [18]. Each sediment (1 g) was mixed with Tenax (0.5 g, TA 20/35) in a 50-ml glass tube with 40 ml of 0.01 M $CaCl_2$ and 0.1% of NaN_3. The mixture was shaken for 24 h and centrifuged for 15 min at 2,000 rpm. The Tenax was transferred to another test tube and extracted twice with 5 ml of hexane. The extract was concentrated to 1 ml under a gentle nitrogen stream for chemical analysis as described earlier.

Toxicity bioassay

The toxicity of pore-water samples was assessed by the luminescent bacterium *E. coli* HB101 pUCD607 lux biosensor assay [6,28]. The luminescent bacterium *E. coli* HB101 is marked with the lux CDABE genes, isolated from *Vibrio fischeri,* using the multicopy plasmid pUCD607. Freeze-dried cultures were reactivated by resuspending the cells in 10 ml of 0.1 M KCl and incubating at 25°C for 1 h on a shaker. Aliquots (900 ml) of each pore-water solution were transferred to luminometer cuvettes, and a 100-ml aliquot of the resuscitated cells was added and mixed into each sample at 15-s intervals. The luminescence of the samples was measured after a 15-min exposure period using a luminometer. On completion of the measurement, the relative light unit value was recorded. The assays were carried out in triplicate, and averages were reported. The luminescence of the samples was expressed as a percentage of the luminescence of the control samples (0.1 M KCl solution).

Concentration–response curves for quality control were derived using Zn standards (i.e., 0, 0.05, 0.1, 0.15, 0.2, 0.3, 0.5, and 1.0 mg/L). These standards were used as interassays to verify the consistency of the method. The median effect concentration was calculated using a calculator program developed by Australia's Commonwealth Scientific and Industrial Research Organization [29,30]. The median effect concentration is a relative measure and therefore should remain the same even though the absolute number of bacteria (and therefore the maximum relative light unit) differs among assays.

Toxicity identification evaluation

For pore-water samples that were highly toxic, a partial TIE analysis (phases 1 and 2) was conducted to preliminarily determine whether metals, organic contaminants, or both contributed to the toxicity. To remove organics, 3 ml of the pore-water samples were filtered through Alltech high-flow C18 cartridges (1 g, 6 ml) at 5 ml/min, and the filtrate was collected for the luminescent bacterial assay. To remove heavy metals, 3 ml of the pore-water samples were treated for 30 min with Chelex-100 ion exchange resin (Bio-Rad) at a ratio of 1:10 (w/v) to remove heavy metals. The treated water samples were then tested with the luminescent bacterial assay. A decrease in the toxicity of either treated sample compared with the original (untreated) sample indicates that the group of chemicals removed in that sample contributed to the toxicity. The TIE procedures were performed in triplicates, and averages (and their standard deviation) were reported.

Data analysis

Data (both chemical and toxic analysis) were analyzed using single-factor analysis of variance after testing for homogeneity of variances and normality. Post hoc Tukey's tests were conducted where significant differences among sites were found. The level of statistical inference was at 0.05.

RESULTS AND DISCUSSION

Metals in the sediments

Total metal concentrations (including the metals Cr, Ni, Cu, Zn, Cd, and Pb and the metalloid As) in the sediment samples from the SOP ranged from 204 mg/kg at S8 (Northern Water Feature) to 862 mg/kg at S11 (Black Creek), while those outside the boundary ranged from 264 mg/kg at S5 (Silverwater Bridge) to 1,152 mg/kg at S9 (Newington Wharf; Table 2). Of the seven elements analyzed (metals Cr, Ni, Cu, Zn, Cd, and Pb and metalloid As) in the sediments, Zn was found to be highest in concentration, followed by Pb, Cu, Cr, Ni, As, and Cd. Zinc concentrations ranged between 84 and 440 mg/kg at sites within the park and between 140 mg/kg and 618 mg/kg at sites outside the park. The mean concentrations for Pb, Cu, Cr, Ni, and As were 82, 47, 40, 23, and 15 mg/kg, respectively, at sites within the SOP and 104, 85, 60, 24, and 19 mg/kg at sites outside the park. The highest concentrations for Zn, Cr, Cu, Pb, and As were found in the sediments of S9 (Newington Wharf). The Australian interim sediment ecological trigger values, which, if not exceeded, correspond to a low probability of biological effects, occurring for Cr, Cu, Pb, Zn, Ni, As, and Cd are 80, 65, 50, 200, 21, 20, and 1.5 mg/kg, respectively [31]. Chromium and Cu concentrations in

Table 2. Concentrations of heavy metals in the sediments of Sydney Olympic Park (SOP), Australia (i.e., S2, S3, S6–S8, S10, S11), and its surrounds (i.e., S1, S4, S5, S9)[a]

			Concentration (mg/kg sediment)							
		Site	Cr	Cu	Pb	Zn	Ni	As	Cd	Total
Non-SOP	UNR	S1	31	36	64	246	22	19	<5	419
		S4	82	62	124	343	23	22	<5	656
		S5	31	41	41	140	<10	11	<5	264
		S9	196	102	188	618	26	23	<5	1,152
SOP	Remnant	S2	31	34	66	243	22	17	<5	412
		S3	37	49	70	250	29	13	<5	449
		S7	36	46	79	397	24	19	<5	601
	R	S6	23	28	35	150	18	13	<5	267
		S8	15	20	85	84	<10	<10	<5	204
		S10	29	55	59	226	23	11	<5	403
		S11	107	94	181	440	22	18	<5	862
		ISQG-low	80	65	50	200	21	20	1.5	

[a] ISQG-low = Australian interim sediment ecological trigger value; R = remediated sites; UNR = unremediated sites.

sediments within the SOP exceeded the trigger values at S11 (Black Creek), while Pb concentrations exceeded the trigger values at all sites except for S6 (Eastern Water Quality Control Pond). Zinc and Ni concentrations exceeded the trigger values at five sites, while the concentrations of As at all sites were below the trigger values (Table 2). Among the unremediated sites (S1, S4, S5, and S9) outside the park, S9 (Newington Wharf) was most contaminated, with essentially all elements exceeding their respective trigger values except for Cd, which could not be determined (Table 2).

The sites outside the park—S1 (Homebush Bay), S4 (Haslams Mouth), S5 (Silverwater Bridge) and S9 (Newington Wharf)—exhibited comparatively higher metal concentrations in their pore water than did sites within the park in most cases (Table 3). The mean concentrations for Zn, Cu, Ni, As, Pb, Cr, and Cd in the pore water were 14, 2.4, 2.8, 1.9, 0.9, 1.1, and less than 0.4 μg/L, respectively, at the sites within the SOP, and 144, 13, 9.3, 4, 1.2, 1.1, and 1.5 μg/L at the sites outside the park. The concentrations for Zn at the sites outside the SOP varied between 37 and 230 μg/L.

Organic contaminants in sediments

The concentrations of dioxins (PCDD/Fs) in the sediments from the SOP varied widely from 17 pg I-TEQ/g at S8 to 131 pg I-TEQ/g at S6 (Table 4). The four unremediated sites outside the park had dioxin concentrations greater than 200 pg I-TEQ/g in the sediments (Table 4). The concentration (1,883 pg I-TEQ/g sediment) at S1 (Homebush Bay) is among the highest dioxin concentrations reported globally [32,33].

The concentrations for PCBs in the sediments within the SOP varied between 14 and 33 μg/kg, with a mean concentration of 21 μg/kg, while the concentrations in the unremediated sites outside the park ranged between 14 and 124 μg/kg, with a mean concentration of 78 μg/kg (Table 4). Two of these sites outside the park (S1 and S4) had concentrations greater than 100 μg/kg. The highest concentration of DDTs (DDT and its metabolites, dichlorodiphenyldichloroethane and dichlorodiphenyldichloroethylene) was also found at S1 (113 μg/kg), followed by S4, S9, and S5 (Table 4), which are all located outside the SOP. The mean concentrations for DDTs in the sediments were 10 μg/kg within the SOP and 66 μg/kg outside the park.

Polycyclic aromatic hydrocarbons were also found in the sediments, with concentrations ranging from 1,146 to 14,461 μg/kg and a mean concentration of 4,538 μg/kg at the sites within the SOP. The concentrations of PAHs in the sediments of the sites outside the park ranged from 3,701 to 13,085 μg/kg, with a mean concentration of 9,688 μg/kg. Four sites had PAH concentrations greater than 10,000 μg/kg, one of which (S6) was within the park (Table 4). Similar distribution patterns for 16 PAHs were recorded for most of the 11 sites analyzed (Fig. 2). The phenanthrene to anthracene (Ph:Ant) ratios ranged from 0.69 to 5.89, while the benzo[*a*]anthracene to benzo[*a*]anthracene plus chrysene (B*a*A:(Ch + B*a*A)) ratios

Table 3. Concentrations of heavy metals in the pore water of sediments from Sydney Olympic Park (SOP), Australia (i.e., S2, S3, S6–S8, S10, S11), and its surrounds (i.e., S1, S4, S5, S9)[a]

			Concentration (μg/L pore water)							
		Site	Cr	Cu	Pb	Zn	Ni	As	Cd	Total
Non-SOP	UNR	S1	0.95	9	1.4	222	8.6	4.6	<0.4	247
		S4	0.95	12	1.1	37	7.1	2.5	0.92	61
		S5	1.8	16	1.7	88	6.4	5.6	1.5	122
		S9	0.83	16	0.55	230	15	3.2	2.2	269
SOP	Remnant	S2	0.66	5.2	0.89	26	2.5	1.7	<0.4	37
		S3	1.6	0.88	0.82	<0.4	2.5	4.4	<0.4	10
		S7	<0.4	0.41	<0.4	24	4.8	0.72	<0.4	30
	R	S6	<0.4	2.4	<0.4	6.4	1.7	0.63	<0.4	11
		S8	<0.4	7.1	1.4	8.5	3	2.1	<0.4	22
		S10	<0.4	0.58	<0.4	4.5	2	0.74	<0.4	8
		S11	<0.4	0.43	0.54	12	3.3	3.2	<0.4	20

[a] R = remediated sites; UNR = unremediated sites.

Table 4. Concentrations of organic contaminants in the sediments of Sydney Olympic Park (SOP), Australia (i.e., S2, S3, S6–S8, S10, S11) and its surrounds (i.e., S1, S4, S5, S9)[a]

				Organic carbon	Concentration (pg I-TEQ/g)	Concentration (µg/kg sediment)		
		Site	pH	(%)	Dioxins	PCBs[b]	DDTs[c]	PAHs[d]
Non-SOP	UNR	S1	6.98	3.5	1,883	112	113	11,714
		S4	7.74	3.9	423	124	66	13,085
		S5	7.97	0.6	374	14	19	3,701
		S9	7.38	3.1	207	62	64	10,251
SOP	Remnant	S2	8.17	3.5	98	15	8	2,550
		S3	8.04	6.7	123	14	9	5,336
		S7	6.33	5.7	ND	24	9	2,905
	R	S6	7.89	2.8	131	21	13	14,461
		S8	8.09	0.3	17	14	10	1,960
		S10	7.60	10.8	64	23	4	1,146
		S11	7.83	6.9	54	33	15	3,409
		ISQG-low[e]			—	23	1.6	4,000

[a] I-TEQ = international toxic equivalency; ND = not detected; PCBs = polychlorinated biphenyls; R = remediated sites; UNR = unremediated sites.
[b] PCBs is the sum of PCB congeners 28, 52, 101, 81, 77, 118, 153, 138, 126, 180, and 160.
[c] DDTs is the sum of *p,p′*-DDT, *p,p′*-dichlorodiphenyldichloroethane, and *p,p′*-dichlorodiphenyldichloroethylene.
[d] PAHs is the sum of 16 U.S. Environmental Protection Agency polycyclic aromatic hydrocarbons.
[e] ISQG-low is the Australian interim sediment ecological trigger value.

ranged from 0.42 to 0.62. A phenanthrene to anthracene (Ph:Ant) ratio of less than 10 usually indicates the source to be from combustion [34]. The B*a*A:(Ch + B*a*A) ratio of less than 0.2 usually implies the source is from petroleum based activities, 0.2 to 0.35 indicates either petroleum or combustion sources, while more than 0.35 denotes combustion sources [35]. The ratios of Ph:Ant were all less than 10 and the ratios of B*a*A:(Ch + B*a*A) greater than 0.35 in all samples investigated, suggesting PAHs in the sites were from combustion sources.

Concentrations of bioavailable fractions of organic contaminants in sediments can be determined from Tenax extracts [18,36,37]. Only PAHs were detected in these extracts, as the other organic contaminants were at relatively low concentrations. The PAH concentrations ranged from 233 to 854 µg/kg, which accounted for 2 to 17% of PAHs in the sediments (Table 5). Relatively higher concentrations were found in the sediments from the unremediated sites—S1, S4, S5, and S9—which also exhibited high dioxin concentrations. Relatively high bioavailable rates (12 and 13%) for the sediments of S5 and S8 could be due to their low organic carbon contents, but this could not explain the case of S10.

The Australian interim sediment ecological trigger values for PCBs, DDTs, and PAHs are 23, 1.6, and 4,000 µg/kg [31]. The concentrations of these contaminants in sediments from the SOP exceeded the trigger values at all seven sites for DDTs, at two sites for PCBs, and two sites for PAHs (Table 4). In the

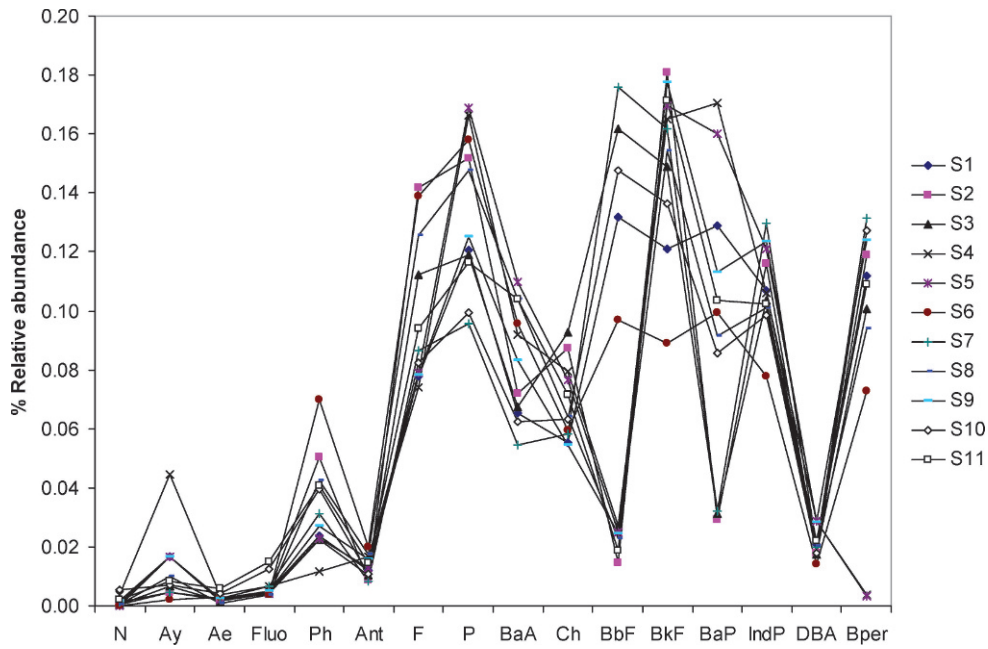

Fig. 2. Polycyclic aromatic hydrocarbons (PAH) profiles for sediments from Sydney Olympic Park, Australia. Ae = acenaphthene; Ant = anthracene; Ay = acenaphthylene; B*a*A = benzo[*a*]anthracene; B*a*P = benzo[*a*]pyrene; B*b*F = benzo[*b*]fluoranthene; B*k*F = benzo[*k*]fluoranthene; Bper = benzo[*ghi*]perylene; Ch = chrysene; DBA = dibenzo[*a,h*]anthracene; F = fluoranthene; Fluo = fluorene; IndP = indeno[1,2,3-*cd*]pyrene; N = naphthalene; P = pyrene; Ph = phenanthrene. See the Figure 1 legend for site numbers.

Table 5. Bioavailable concentrations of total polycyclic aromatic hydrocarbons (PAHs) in the sediments from Sydney Olympic Park (SOP), Australia (i.e., S2, S3, S6–S8, S10, S11) and its surrounds (i.e., S1, S4, S5, S9)[a]

		Site	Total PAH concentration in sediment (μg/kg)	Total PAH concentration in Tenax (μg/kg)	% Bioavailable
Non-SOP	UNR	S1	11,714	854	7
		S4	13,085	683	5
		S5	3,701	447	12
		S9	10,251	648	6
SOP	Remnant	S2	2,550	233	9
		S3	5,336	325	6
		S7	2,905	290	10
	R	S6	14,461	257	2
		S8	1,960	248	13
		S10	1,146	192	17
		S11	3,409	347	10

[a] R = remediated sites; UNR = unremediated sites.

unremediated sites outside the park, the concentrations of PCBs, DDTs, and PAHs exceeded the trigger values at three, four, and three sites, respectively (Table 4). The DDT was manufactured by Orica (previously Union Carbide) on the eastern shore of Homebush Bay, which could be the source of the high concentrations measured in the sediments. Bioactivity of persistent organic pollutants and their effects in mosquitofish collected from the SOP were discussed in another investigation [38].

Toxicity assessment

Pore-water samples were used to assess toxicity of the sediments using the *E. coli* HB101 pUCD607 lux biosensor. Significantly decreased luminescence ($p < 0.05$) occurred with the four pore-water samples from the unremediated sites (S1, S4, S5, and S9) outside the park (Fig. 3), suggesting the presence of chemicals at concentrations that are toxic to the bacteria. The pore-water samples from the seven study sites within the park had higher (suggesting hormesis) or similar luminescence values to that of the control (0.1 M KCl solution). These findings are consistent with the results from chemical analysis and the Tenax bioavailability study. The contaminants in the sediments of the remediated sites of the SOP exist at levels that are not toxic to the test bacteria. The four unremediated sites outside the park were contaminated by dumping of industrial chemicals containing dioxins and others. The high toxicity to *E. coli* is due to the high levels of toxic chemicals in the sediments. Unfortunately other environmental factors such as total organic carbon could not explain the observed chemistry and toxicity patterns.

Toxicity identification and evaluation

Toxicity identification and evaluation manipulations were applied to the pore-water samples that showed significant toxicity (S1, S4, S5, and S9; Fig. 3) using metal-chelating resin and C18 solid-phase extraction cartridges. After treatment with metal-chelating resin, the relative light unit values were essentially the same as the untreated pore water, suggesting metals did not significantly ($p > 0.05$) contribute to toxicity in the samples (Fig. 4). In contrast, after elution through C18 solid-phase extraction cartridges, pore-water samples from two unremediated sites (S4, Haslams Mouth, and S5, Silverwater Bridge) showed significantly higher ($p < 0.05$) luminescence values than that of the control, confirming that organic compounds were indeed responsible for toxicity in the pore-water samples from these two sites. The C18 cartridges can

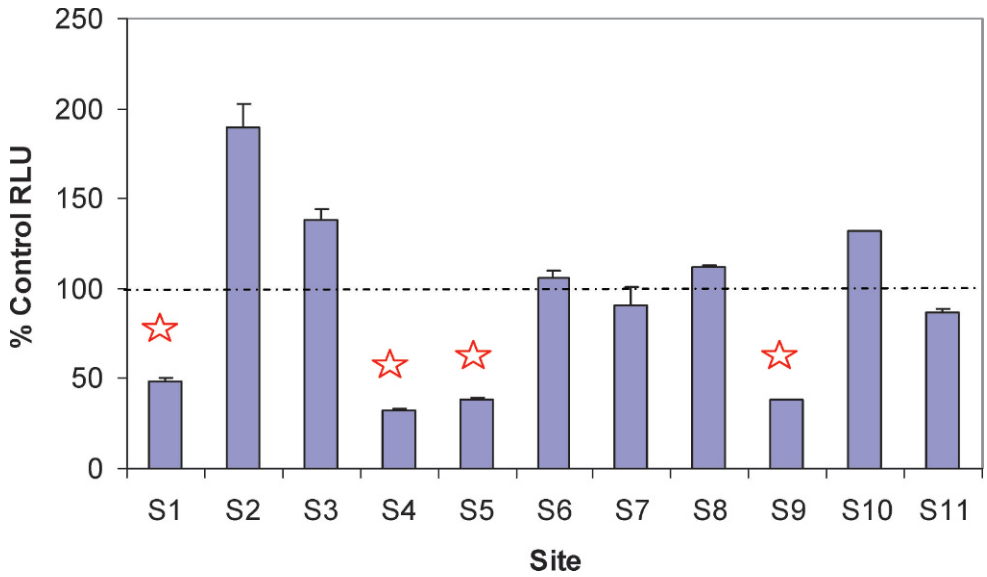

Fig. 3. Luminescence measurements as a percentage of the control for the pore-water samples from Sydney Olympic Park, Australia and its surroundings, in relative light units (RLUs). Stars in the graph indicate inhibition of luminescence in comparison with the control. See the Figure 1 legend for site numbers.

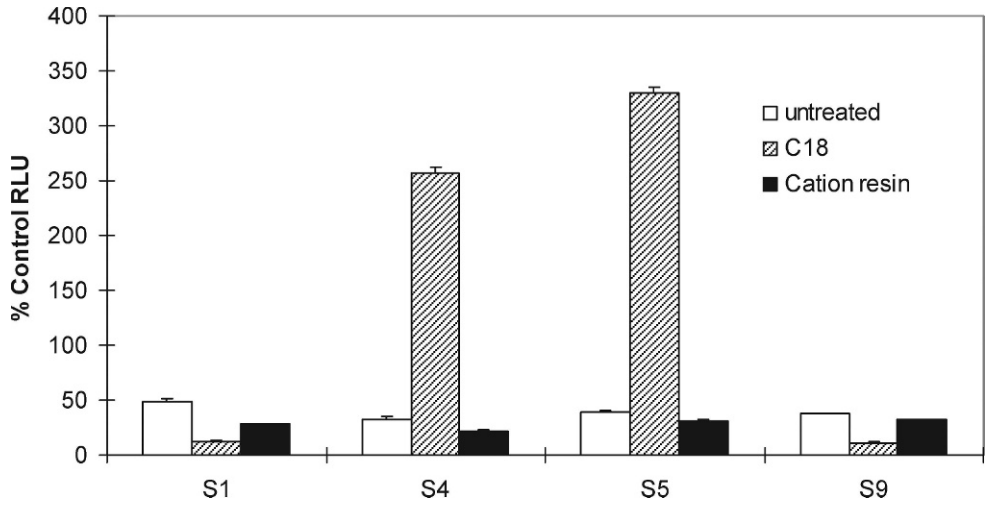

Fig. 4. Luminescence measurements for the pore-water samples from the unremediated sites outside Sydney Olympic Park, Australia, after treatment with C18 cartridges or cation resin. RLU = relative light unit. See the Figure 1 legend for site numbers.

effectively remove organic compounds with a log K_{OW} value greater than 2 [39]. The present study indicates that moderately polar and hydrophobic compounds contributed to the toxicity of the pore water at S4 and S5. However, pore-water samples from S1 and S9 still gave very low relative light unit values (Fig. 4), which suggests that contaminants other than hydrophobic organic compounds (removed by C18 cartridges) are still contributing to the toxicity of the pore-water samples. Nevertheless, the contribution of metals to toxicity cannot be proved due to the lack of reduction of toxicity upon treatment with cation resin. This is surprising given the high total metal concentrations in the pore-water samples from S1 and S9 (246 and 268 μg/L, respectively). Concentrations of the dominant metal cation, Zn, in the pore-water samples were highest at 222 and 230 μg/L for S1 and S9, respectively, compared with only 37 and 88 μg/L for S4 and S5, respectively. These concentrations for S1 and S9 were much higher than the measured median (25 μg/L) and 10% (9 μg/L) effect concentration values for the luminescent bacteria to Zn.

Other factors in the sediments, such as sulfide and ammonia, could contribute partially to the toxicity of the pore-water samples [14,17,40]. Such circumstances are fairly common in fine-grained anaerobic sediments. However, ammonia and sulfide are nonpersistent compounds that easily transform to nontoxic nitrate and sulfate when samples are aerated [16]. Ammonia could be a toxicant in the sediments; however, luminescent bacteria are not sensitive to ammonia as previously reported by Ankley et al. [41]. Thus, ammonia in the pore water of the sediments is not likely to be a significant toxicant to the luminescent bacteria. The low pore-water concentrations of Zn for S4 and S5 samples may be due to presence of sulfide and anoxic conditions in sediments. Field-measured redox potentials of samples from the four sites were 19 and 3 mV at S1 and S9 in contrast to −82 and −84 mV at S4 and S5, respectively. The positive field-measured redox potentials values at S1 and S9 suggest the absence of sulfate reduction, with very low sulfide concentration; consequently, the elevated pore-water concentrations were expected to have contributed to the toxicity to the lux biosensor. Unfortunately, the physiochemical conditions of these pore-water samples could be influenced by experimental procedures, although the pore-water samples were not aerated deliberately.

The ineffectiveness of chelating resin treatment to completely remove the metals from the samples cannot be ruled out as one of the reasons for lack of reduction of toxicity in samples from S1 and S9. In any case, organic chemicals (and possibly metals for S1 and S9) were the predominant toxicants in pore water of the sediments from the four unremediated sites outside the park.

CONCLUSION

Pore-water toxicity tests with the luminescent biosensor identified 4 (Homebush Bay, Haslams Mouth, Silverwater Bridge, and Newington Wharf) of the 11 sites as having clear toxicity effects. This is generally consistent with the bioavailable fractions (pore-water and Tenax extraction data), as well as dioxin concentrations, in the sediments. The four sites are located outside the park in Homebush Bay and Parramatta River and have not been remediated. Preliminary TIE tests demonstrated that organic contaminants were the main cause of toxicity to *E. coli,* with metals not contributing to the toxicity in the pore water. In contrast, the sediments and their pore water from sites within the park were not toxic to the luminescent bacteria. The present study clearly demonstrates the successful application of combined approaches of chemical analysis and biosensor assays to assess contaminated sites to prioritize sites for remedial action.

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